

EFFECTS OF TEMPERATURE AND PHOTOPERIOD ON EMBRYONIC DIAPAUSE  
IN *NEMOBIUS FASCIATUS* (DE GEER) (ORTHOPTERA, GRILLIDAE)

DARSHAN SINGH SARAI  
Department of Agriculture  
Courthouse, Kelowna, B.C.

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The effects of temperature and photoperiod on invoking and terminating embryonic diapause of *Nemobius fasciatus* were studied in relation to the adaptation of this cricket to the long winter of a continental climate at 53 40' N.

*N. fasciatus* is univoltine in Alberta. Eggs undergo facultative diapause at the termination of anatrepsis, overwinter, and hatch in early July of the succeeding year. There are six nymphal instars; adults appear early in August and survive until frosts occur in late October. Diapause appears to be induced mainly by the wide diurnal temperature range in late summer. Middle aged females deposit higher percentages of diapausing eggs than younger or older females. A high incubation temperature (29°C) suppresses diapause. Diapause is terminated by exposure of pre-diapausing or diapausing eggs to temperatures of -15°C before incubation. Heavy mortality occurs, however, in freshly laid eggs when they are exposed to 0°C. A temperature of 5°C halts development of post-diapause eggs but this is resumed within 24 hours at an incubation temperature of 29°C. Exposure of any stage to constant or varying photoperiod does not apparently influence the incidence of diapause.

A single generation per year and resistance to low temperature, at least in the resting stages, are commonly found in insects which inhabit high latitudes. In these regions climatic variations which are responsible for these adaptations are very wide. In the resting stage, developmental processes and metabolism are very much retarded. This phenomenon of physiological rest or diapause, is followed by an active period of the life cycle at the onset of favourable conditions. Danilyevsky (1965) attributes the univoltine life cycle to the short duration of favourable conditions. Where favourable conditions continue longer, insects are mostly multivoltine. Diapause in univoltine insects is mostly obligatory and in multivoltine species facultative (Andrewartha and Birch 1954, Lees 1955, Danilyevsky 1965). In the former, diapause is commonly invoked by internal physiological processes regardless of the external conditions, but the life cycle as a whole remains under the influence of temperature which determines the breaking of diapause and also the duration of development of the active stages. In multivoltine insects facultative diapause is mainly controlled by external factors, such as photoperiod, temperature, food, and humidity (Lees 1955, Danilyevsky 1965).

It was once believed (Readio 1931, Cousin 1932) that diapause was invoked by unfavourable conditions in most insects. But the principal stimulus for the inception of diapause is photoperiod, although temperature, water, and diet may be involved (Harvey 1962). Although photoperiod has no direct effect on the development of insects, it may, through the neurosecretory system, induce diapause immediately prior to unfavourable conditions. The discovery of the dependence of the inception of diapause on photoperiod was mainly the work of Kogure (1933) who studied bivoltine races of *Bombyx mori* L. in Japan. Recent works include those of Danilyevsky in 1948, 1949, and 1951, (cited in Danilyevsky 1965), Dickson (1949), Lees (1955), and Corbet (1956).

The present study was undertaken to determine how *Nemobius fasciatus* (DeGeer), the striped ground cricket, is adapted to inhabit one of the coldest locations in its distribution. As this adaptation is mainly through embryonic diapause, this study is concerned with the effect of environmental factors on induction and termination of diapause. This species is omnivorous, as are most crickets, feeding on decaying organic matter and leaves of grasses. Diet was discarded as a possible environmental factor affecting diapause because crickets are general feeders. Similarly water was eliminated, because eggs remain in moist soil from the time of oviposition to hatching. Temperature and photoperiod were, therefore, studied in various combinations.

Crickets were collected in 1964 in hummocky pastures near Atim Creek 21 miles west of Edmonton city centre (53°40'N, 113°50'W). In 1965, another meadow two and a half miles from Atim Creek was used because the previous field was flooded. Crickets break their metathoracic legs easily if netted and to collect them uninjured the mouth of a wide-mouthed fruit jar was put in front of adults and last instar nymphs which were induced to jump into it. I sometimes collected 80 crickets in an hour. It was easy to locate males by their sound. Each male was commonly found with one or two females. Young nymphs were collected in the field with an aspirator. Crickets brought from the field were kept in the laboratory in battery jars with rabbit pellets for food (Ghoury and McFarlane 1958), inverted bottles of water plugged with absorbent cotton for water supply, and strips of paper towels to increase the surface area for walking and for hiding. About 20 to 30 crickets in sex ratio of one were kept in each jar. Fresh moist soil in plastic petridishes was provided for oviposition each 24 hours. Eggs were sieved out underwater in a tray, damaged and small ones were discarded, others were put in plastic petri dishes on a filter paper covering a thick pad of wet absorbent cotton. These petri dishes were kept covered except for a few minutes every day for observation. Eggs are highly susceptible to desiccation. The cotton remained moist for about three weeks at 29 and 24 C and for two months at 0, 5, and 15 C. A few drops of tap water were added when needed. Dishes were marked on the cover and on the under surface with the number of the experiment, the number of eggs, and the date of oviposition.

Eggs were observed after 24 hours for hatching. Newly hatched nymphs were removed from the dish with an aspirator and counted as they were transferred to a fruit jar. Egg shells were removed and counted to check this.

Eggs for laboratory experiments were deposited by the crickets which were collected in the field mainly in the first half of September, when ovipositing adults were abundant. Eggs laid within 24 hours of the collection of the crickets were used. Unless otherwise stated the experiments of 1964 were repeated under similar conditions in 1965, on comparable dates.

Hogan's method (1959) for the Australian *Acheta commodus* Walker was used to determine the stage of embryonic development at which diapause occurs. The eggs were soaked in water in a watch glass for half an hour. The water was then replaced by a mixture of two parts of glacial acetic

acid, two parts of chloroform, and one part of absolute alcohol, for 25 minutes at 34 C. Eggs were then transferred to a mixture of one part of glycerol and one part of 70 per cent alcohol. The embryo was then clearly observed under the microscope without cutting sections or dissecting eggs.

The percentage hatching of eggs shown in the results includes only those which hatched within 13 to 15 days at 29 C or within 20 to 22 days at 24 C, for non-diapausing or post-diapausing eggs. Throughout this study these were the periods required for development without diapause at these incubation temperatures. Diapause in many eggs results in a prolonged hatching period. A typical curve of percentage hatching of total eggs versus incubation days at 29 C is illustrated in fig. 1. Unless otherwise indicated the limits of variation of controlled temperatures throughout this work were less than  $\pm 1$  C.

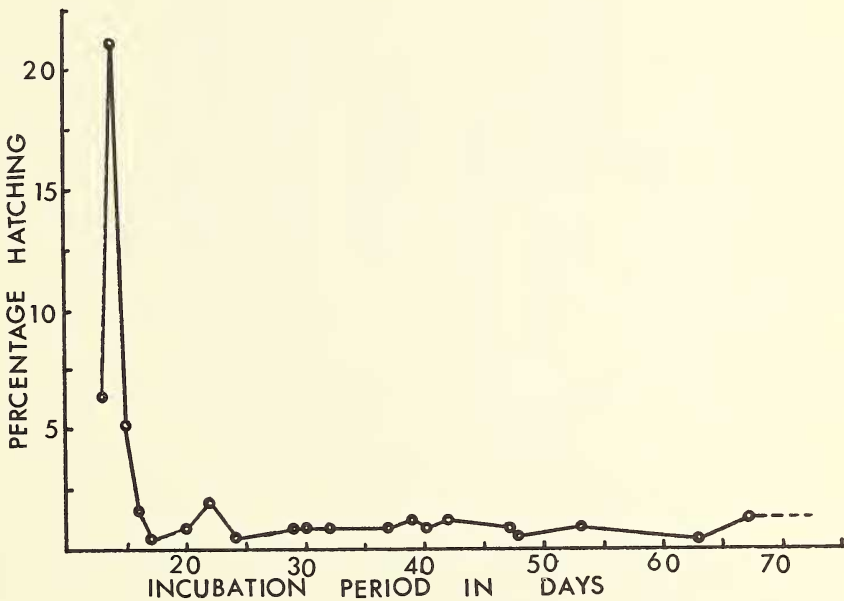


Fig. 1. Frequency distribution of hatching of eggs of *N. fasciatus* at an incubation temperature of 29 C. N = 250.

The crickets from the vicinity of Edmonton were very kindly determined by Dr. R.D. Alexander to whom eight male and female specimens with notes on habitat and a tape recording of the song were sent for this purpose.

## DEVELOPMENT IN THE FIELD

*N. fasciatus* is recorded in wet localities over most of eastern North America including Newfoundland and Florida. By putting together various records of its distribution (Lugger 1897, Blatchley 1920, Fulton 1934, Hebard 1930, 1936, Strohecker 1937, Ball *et al.* 1942, Alexander and Thomas 1959, Vickery 1963) it is found that this species is distributed from southern Canada to northern Mexico and east of the great basin in the United States. Characteristically, it inhabits marsh borders and other poorly drained grassy situations such as stream banks, meadows and pastures. It is commonly associated with crab grass (*Digitaria sanguinalis* Scop.).

In the northern United States this cricket overwinters in the egg, matures in July, and continues to sing until the last individuals are killed by winter. In Ohio, the earliest recorded singing date is July 8 and the latest November 14. In the University of Michigan Museum there are adult specimens from central and northern localities of Michigan, the earliest of which was collected on July 17, the latest on November 16. There are also adult specimens which were taken in Florida every month of the year (Alexander and Thomas 1959). *N. fasciatus* starts maturing before the middle of June in the Piedmont area and coastal plains of North Carolina and probably there are two to three generations per year at this latitude (Fulton 1934). Near Edmonton in 1964 and 1965 the song of this cricket was not heard before the first week of August or after the last week of October.

**Seasonal History at Edmonton**

The seasonal history of *N. fasciatus* was studied in meadows near Atim Creek from the end of April to the end of October in 1964 and 1965. In 1964 eggs hatched from July 8 to 15, adults were first seen on July 30, but there was no singing although the day was warm (22.7 C) and singing starts a day or so after the last molt. A week later, the buzzy chirps were very noticeable. Oviposition had started by August 10, and reached a maximum between the last week in August and the middle of September (fig. 2). There was heavy oviposition on warm and particularly on sunny days, especially when this fine weather continued for two or more days. Below 15 C there was little or no oviposition even in September. Fifth and 6th instar nymphs were seen for the last time on October 10. Heavy frosts in the third week of October killed most of the crickets and they were heard last on October 24, a partly sunny day with afternoon temperature 11.6 C.

In 1965 there were 7.48 inches of rainfall in June compared to 1.04 inches in 1964 and 3.15 inches normal for this month. Meadows near Atim Creek were flooded from June 21 to August 10 and muddy until the middle of September; plant growth reappeared by the end of the month. Neither crab grass nor crickets were present. To test the viability of eggs deposited here in 1964, soil samples were taken in October 1965 from sites where oviposition had been observed. No eggs sorted from the mud by sieving under water hatched. Seasonal life history was observed in the unflooded edge of a spruce grove to the north and in another



meadow about 2.5 miles away which dried early. It was similar to that of 1964; singing was first heard on August 10, some adults were present but 5th and 6th instar nymphs predominated. On September 24-25 0.9 inches of snow fell and stayed for a day and a half. This snow and nightly frosts from September 22 to 28 killed most of the crickets. Singing was last heard on October 13, a sunny day with afternoon temperature 12.2 C. The adult population was maximum by the end of August in each year.

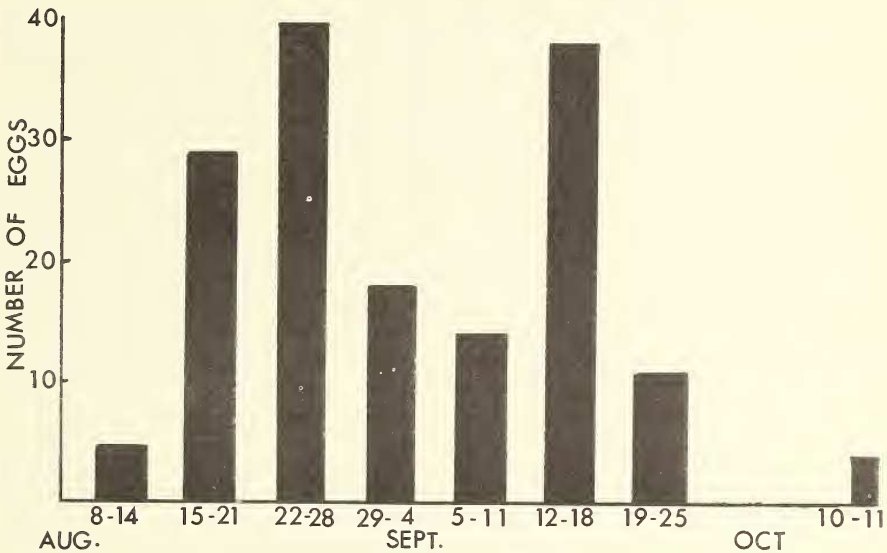


Fig. 2. Number of eggs deposited by *N. fasciatus* per week per female caged outside, 1964.

#### Seasonal Incidence of Diapause

Almost synchronous hatching of *N. fasciatus* in July, and overwintering of the egg stage suggest a regulating mechanism by which the seasonal rhythm of development is adapted to this climate. This mechanism could be the changes in photoperiod or daily temperature during late summer. An experiment was conducted to determine whether the changing photoperiod or temperature influenced the females to lay diapausing eggs.

#### Methods and materials

Adults were collected weekly from August 7 to October 10, 1964 and kept in battery jars in screen cages of two cubic feet volume outside under natural conditions of photoperiod and temperature. The locations of the cages were selected to avoid artificial light and shadows from buildings or trees. Wet soil in plastic petri dishes was supplied for oviposition and renewed every 24 hours. Throughout the experiment eggs were sieved out in water and incubated at 29 C in petri dishes. In all 4,644 eggs were

incubated at this temperature.

Three other batches of five hundred and fifty eggs each were incubated at 29 C, 22 C, and  $20 \pm 1.5$  C respectively. Lots of about 100 were started one week apart from August 16 to September 20. Temperatures in the cages were recorded with thermographs. Data for Edmonton photoperiods for the complete year were obtained from the Dominion Public Weather Office, Edmonton.

#### Results

The percentage hatches (within 15 days) of eggs deposited during succeeding weeks of the summer of 1964 and incubated at 29 C are shown in Table 1. These results show no obvious correlation between the incidence of diapause and any weather factor, except the difference between day and night temperatures or amplitude of temperature variation. There was over 50 per cent diapause whenever the amplitude of temperature difference was greater than 16 C. Either the eggs within the female are directly sensitive to changes in environmental temperature, or they are influenced indirectly through some physiological mechanism in the female that is affected by fluctuating temperatures. It is apparent from Table 1 too that in the first halves of August and October the number of diapausing eggs is less than in September. Thus a second factor contributing to the percentage of diapausing eggs could be the age of females.

TABLE 1. The percentage hatch of eggs deposited in successive weeks by *N. fasciatus* under natural conditions of temperature and photoperiod and incubated at 29 C, 1964.

Week	Mean temp. C	Mean photoperiod		Temperature range C and amplitude	Per cent of diapausing eggs
		hr.	min.		
Aug. 8-14	20.5	15	12	15.5-27.7, 12.2	36.8
Aug. 15-21	18.8	14	42	10.0-26.7, 16.7	72.4
Aug. 22-28	15.0	14	15	7.2-26.7, 19.5	75.1
Aug. 29-Sept. 4	12.2	13	47	7.2-20.0, 12.8	53.3
Sept. 5-11	9.3	13	17	-1.0-23.0, 24.0	65.2
Sept. 12-18	12.8	12	48	3.3-25.5, 22.2	63.0
Sept. 19-25	16.6	12	19	2.2-25.0, 22.8	78.4
Sept. 26-Oct. 2	8.8	11	49	1.7-15.5, 13.8	29.3
Oct. 3-9	12.5	11	20	1.7-25.5, 23.8	59.6
Oct. 10-11	7.2	11	02	0.6-15.5, 14.9	28.7

In the batches at different incubation temperatures the overall percentage hatch in 15 days at 29 C was 35.6. At 22 C no hatching occurred within 25 days, 3.5 per cent hatched in 60 days and 4.3 per cent in 90 days. At 20 C there was only 3 per cent hatch in 90 days. Thus all the eggs incubated at 22 C or 20 C diapaused. It can be concluded that continuous higher incubation temperatures suppress diapause. No significant

differences due to starting dates were found.

### Discussion

Differences in the dates of the first and last singing in Ohio, Michigan, and Edmonton are probably due to the difference in temperatures at these places. North Carolina is warmer than Ohio and Michigan, and two to three generations per year are possible (Fulton 1931). In Florida the temperature remains fairly high throughout the year and the variation in the mean monthly temperatures for the years 1941-50 (World Weather Records 1959), is only between 11.4 and 27.7 C, which allows this cricket to breed throughout the year. Fig. 3 shows a comparison of mean monthly temperatures at four localities with the type of life cycle at these places. It appears that the number of generations per year at different latitudes is in fact determined by the period of high temperature available.

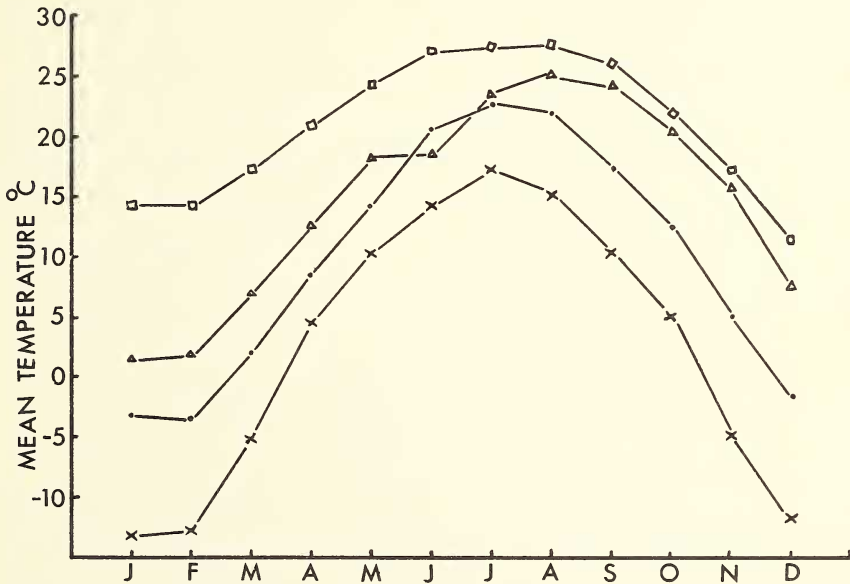


Fig. 3. Mean monthly temperatures (C) at different localities in relation to voltinism in *N. fasciatus*. ■ Jacksonville, Florida - Multivoltine; ▲ Cincinnati, Ohio - Probably univoltine; • Detroit, Michigan - Probably univoltine; × Edmonton, Alberta - Univoltine.

Since the mean maximum temperature in Edmonton during August, September, and October rarely goes above 22.5 C there is no hatching of eggs deposited earlier in the season which is in accordance with the laboratory results (4.3 per cent hatch in 90 days at 22 C). A few nymphs which were seen in the late summer probably hatched late from the previous year's eggs or were exceptionally slow in developing. This point was cleared up during the study of the life history in the laboratory where some nymphs developed exceptionally slowly and some died before reaching maturity.

Cragg and Cole (1952) observed that there were very high fluctuations in the number of non-diapausing larvae in the blowfly, *Lucilia sericata* Meig. throughout the summer months. They stated (p. 603) that there was no obvious correlation between the fluctuations and any one weather factor. The same appears to be true of *N. fasciatus*, so far as photoperiod, number of eggs laid, or hours of bright sunshine are concerned. Fluctuations in the number of diapausing eggs laid during succeeding summer months were apparently due to the range of temperatures experienced by the females during the time of oviposition. Crickets which were exposed to a range of temperatures favourable for embryogenesis (15.5 to 27.7 C), or favourable for breaking diapause (0.6 to 15.5 C) laid less diapausing eggs. A large number of diapausing eggs were laid by crickets exposed to widely fluctuating temperatures (below 10 to above 20 C).

Many articles have been published which deal with the effect of seasonal fluctuations in temperatures on the induction and termination of diapause. It is generally accepted that diapause termination in most insects needs low temperatures whereas embryogenesis in post-diapause stages requires high temperatures to proceed. There has been, however, very little work done on the effects of daily fluctuations of temperature on diapause. Kozhanchikov (1949) associated diapause in the Chinese oak silkworm *Antheraea pernyi* Guer. with the effects of daily fluctuations in temperature on the larvae. He found that all of the larvae transformed into non-diapausing pupae when the daily temperature varied from 19 to 22 C at a mean rearing temperature of 20 C. However, with a range of 18 to 25 C at the same mean temperature 86 per cent of the larvae diapaused, and all the larvae diapaused when the daily fluctuations in temperature were from 18 to 32 C, 15 to 30 C or 15 to 32 C. At a slightly lower rearing temperature (17 C) all larvae underwent diapause when the daily temperatures varied from 15.2 to 25.0 C, 14.0 to 25.0 C and 14.0 to 30.0 C. Therefore, in *Antheraea pernyi* Guer. both the mean rearing temperature and the range of fluctuation are important in invoking diapause.

Browning (1952b) observed in *Acheta commodus* Walk. (= *Gryllulus commodus*) that as the incubation temperature is raised, an increasing percentage of the eggs develops without diapause. His observations are confirmed by Hogan (1960a) and also apply to *N. fasciatus*.



## PRELIMINARY LABORATORY STUDY OF DEVELOPMENT

**Effects of Photoperiod and Age on Diapause**

Since the previous experiment did not show any relationship between photoperiod and the incidence of diapause, another experiment was conducted to verify those results by changing the photoperiod in the laboratory while other conditions such as food, humidity, and temperature were controlled. Another reason for this experiment was to determine whether diapausing eggs are deposited in response to changing photoperiod experienced by adults or nymphs.

*Methods and materials*

Last instar nymphs of both sexes were collected on July 29, 1964 and kept in battery jars in four lots of 20. These jars were placed in a growth chamber provided with fluorescent and incandescent lights operated by time switches, and a humidity control. Two groups of these were exposed to changing photoperiod and the other two served as controls kept in constant darkness. The photoperiod corresponding to September 1 in Edmonton was first used in order to break the continuity of the July photoperiod experienced in nature by the nymphs. A light intensity of 500 foot-candles was recorded on the surface of the shelf holding the battery jars. The photoperiod was changed to that of September 6 after five days and similar changes continued until the photoperiod was that of October 28. Temperature was maintained in the chamber at 28.9 C for 12 hours per day and at 1.5 C for the remaining 12 hours. The relative humidity was maintained at 68 per cent. The control battery jars were covered securely with a light proof cover made of plastic which was black on the inside and white on the outside. The crickets matured from July 30 to August 3 and eggs were laid mainly from August 10 to September 23. They were collected every day and incubated at 29 C.

*Results*

As shown in fig. 4 only those eggs which were deposited by crickets when under 12 hr 36 min or 9 hr 49 min photoperiod per day, showed a high percentage hatch (45.9 and 43.3 per cent, respectively) whereas only 15.5 to 29.2 per cent of the rest of the eggs hatched. When these results are compared with those of field adults laying eggs under the same photoperiods, there is no similarity. Changing photoperiod has no apparent relation to the incidence of diapause. Furthermore, the percentage hatch of eggs laid by the illuminated crickets (23.2) is significantly higher at the 1 per cent level than that of eggs laid by the control ones (12.9). This could result from the absorption of radiant heat by the illuminated crickets. In the control groups the difference in percentage hatch of eggs deposited by females of different ages was not significant even at the 5 per cent level. This suggests that light is necessary to the full expression of the tendency of middle-aged females to lay diapausing eggs.

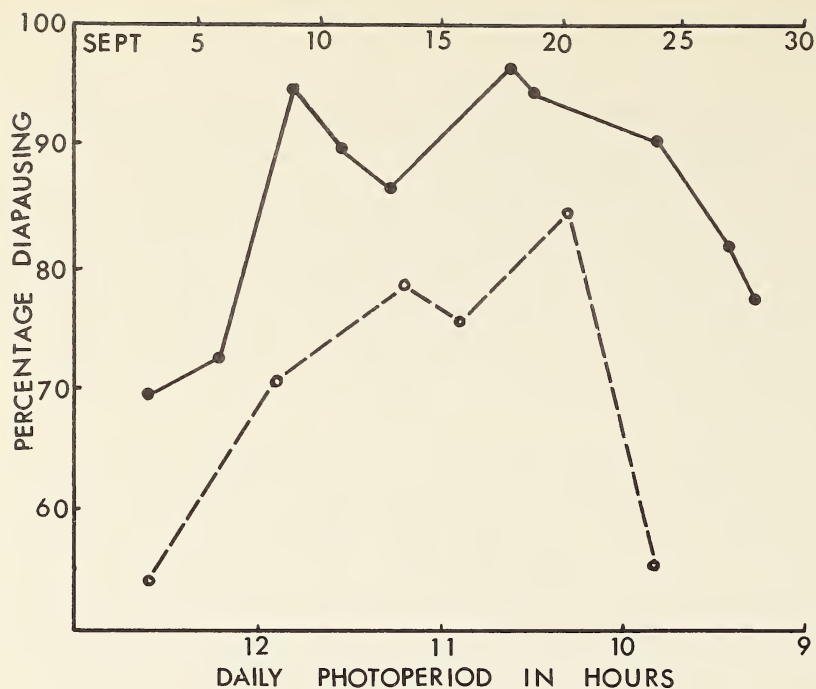


Fig. 4. The percentage of diapausing eggs laid on succeeding dates by crickets kept in continuous darkness (above ●—●) and under decreasing photoperiod (below ○—○) at 28.9 C for 12 hours and 1.5 C for remaining 12 hours per day. Incubation temperature 29 C.

#### Nymphal Development

There is no information concerning the number or duration of nymphal instars in *N. fasciatus* under field conditions or in the laboratory. Nymphal development was studied in the laboratory to permit recognition of stages collected from the field and allow experiments in which different instars were given photoperiod treatments.

#### Methods and materials

Eggs were collected from females kept in the laboratory. They were maintained at 5 C for 30 days to break diapause and then incubated at 29 C or 24 C. Nymphs emerging from eggs on any one day were divided into groups of either ten or two which were kept separately in rearing jars. Forty-five jars were kept at each of the incubation temperatures. Twenty of these contained 10 nymphs each, and the remaining 25 two nymphs each. The jars were covered with fine insect screening and the crickets were individually observed for moulting by removing paper strips and vials every 24 hours from the first instar to the adult stage. For the first three nymphal instars, the light colour of newly moulted nymphs, body size, and width of the head capsule between the eyes were the criteria used to determine new moultings. Exuviae were of little value as they were usually consumed by the nymphs. Nymphs older than the fourth instar were examined for the length of wing pads and ovipositor. Measure-

ments were made with an ocular micrometer.

#### Results

There are six nymphal instars. The mean duration of the nymphal instars and the mean width of the head capsule between the compound eyes are set out in Table 2. The first three nymphal instars are almost of the same duration at each of the two temperatures, whereas, the fourth, fifth and sixth instars differ considerably. There is a difference of about 0.1 mm in the width of the head capsule between each of the first three instars. Only injured and dying nymphs were attacked and eaten by healthy ones. No differences were observed between the two sizes of groups.

TABLE 2. The duration of nymphal instars of *N. fasciatus* in days and the width of the head in mm. Means  $\pm$  standard deviations.

Instar	Duration at 24 C	Duration at 29 C	Head width mm
1st	5.8 $\pm$ 0.38	4.1 $\pm$ 0.47	0.5 $\pm$ 0.02
2nd	5.5 $\pm$ 0.52	3.8 $\pm$ 0.66	0.6 $\pm$ 0.02
3rd	5.7 $\pm$ 0.61	4.0 $\pm$ 0.80	0.7 $\pm$ 0.05
4th	6.3 $\pm$ 0.71	4.4 $\pm$ 0.90	0.9 $\pm$ 0.02
5th	8.0 $\pm$ 1.03	5.2 $\pm$ 0.82	1.0 $\pm$ 0.07
6th	9.8 $\pm$ 1.03	6.6 $\pm$ 0.74	1.2 $\pm$ 0.05
adult	-	-	1.4 $\pm$ 0.09

From field observation and these experiments this species is univoltine in Edmonton. Non-diapausing eggs cannot hatch the same year, firstly because cumulative temperature is insufficient and secondly because the big differences between day and night temperatures induce diapause.

#### Submergence in Water

It was observed previously that two to three days exposure to muddy soil was not detrimental to eggs, while 51 days was. The effect of submergence of eggs in water in the laboratory was, therefore, studied.

#### Methods and materials

Eggs deposited on September 27 and 28 by females collected from Atim Creek meadow in 1964 were used. Four hundred and fifty eggs were kept at 5 C from the end of October 1964 to July 5, 1965 when the following experiment was started. Five groups each of 75 eggs were treated by submerging them under tap water in bottles for 5, 10, 15, 20, and 25 days. A remaining group of 75 eggs was incubated in the normal manner to serve as a control. Each bottle was provided with one inch of wet soil. Eggs were placed a few millimeters below the surface of the soil. The bottles were then filled with tap water gently without disturbing the eggs and kept at 29 C. After treatment eggs were sieved out and incubated at

29 C.

*Results*

Table 3 shows the percentage mortality and the minimum incubation period after immersion. Development seems to continue at about its normal rate at 29 C under water for eight to nine days and then cease. There is a significant correlation at the one per cent probability level between duration of submergence and percentage mortality. Submergence of eggs in water is clearly detrimental after 5 days and about three-quarters of the eggs in the field will be killed by 25 days of flooding. Barber and Dicke (1939) have shown that pupae of *Heliothis armigera* Hubner are killed by water; moreover, mortality increases with a rise of temperature.

TABLE 3. The lethal effect of submergence in water for different periods on eggs of *N. fasciatus*.

Immersion	Minimum additional incubation period in days	Percentage mortality
0	10	15
5	5	27
10	2	32
15	2	39
20	2	57
25	2	72

r (correlation coefficient) between period  
of submergence and percentage mortality = 0.97\*\*  
5% probability level = 0.88  
1% probability level = 0.95

(\*\*  $p < 0.01$ ; \*  $p < 0.05$ )

# EFFECTS OF LOW TEMPERATURES IN THE LABORATORY ON DIAPAUSE

While both temperature and photoperiod may invoke or terminate diapause, Lees (1955, p. 53) stated that temperature is by far the most important environmental agency controlling the termination of diapause. There are many examples of insects from several orders in which diapause is broken by exposure to low temperatures. It has been shown in a number of species of Orthoptera (Parker 1930, Burdick 1937, Andrewartha 1943, Church and Salt 1952, Browning 1952a, b, Hogan 1960a, b, Rakshpal 1962a, b, Masaki 1962) that exposure of diapausing eggs to low temperatures for an optimum time leads to ready development when they are incubated at an appropriately higher temperature.

The effects of continuous low temperatures for different periods and of alternating low and high temperatures on diapausing and post-diapaus-



ing eggs were investigated. In discussing the results of these experiments I have arbitrarily described hatching within three days of first hatch of less than 30 per cent of eggs as indicating full diapause and above 80 per cent as indicating no diapause. Hatching of between 30 and 80 per cent within 3 days is described as partial breakage of diapause.

#### Effects of Low Temperature on Pre-diapause Eggs

Eggs laid in August and September undergo a longer period of high temperatures before being chilled than those laid in October. It has been shown in some orthopteran species (Parker 1930, Church and Salt 1952, Browning 1952a, b) that diapause is broken in eggs which are exposed to low temperature before the diapause stage is reached. But until recently the effect of low temperatures on eggs of different ages has scarcely been explored, except for some work by Hogan (1960b) on *Acheta commodus* Walk., Rakshpal (1962b) on *Gryllus pennsylvanicus* Burm. and Masaki (1962) on the Emma field cricket, *Gryllulus mitratus* (Burm.).

An experiment was carried out primarily to determine the relationship between low temperatures and the breaking of diapause in eggs of various ages. It was also possible to see if low temperatures had any detrimental effect on these eggs. Rakshpal (1962b) observed very high mortality when one day old eggs of *Gryllus pennsylvanicus* Burm. were exposed to low temperature.

#### Methods and materials

About 80 crickets were collected from the field and kept in the laboratory in four rearing jars. Fourteen hundred eggs laid within 24 hours were collected on each of two successive days. Each group of fourteen hundred eggs was divided into lots of 50. Two replicates each of 50 eggs were moved after keeping at 22 C for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 16, and 21 days (referred to as the initial incubation period) to either 0 C or 5 C for 30 days. Thereafter these eggs were incubated at 29 C. Zero days initial incubation meant that eggs which were laid within 24 hours were exposed directly to low temperatures, whereas one day initial incubation meant that eggs laid within 24 hours were kept at 22 C for one further day, and so on.

#### Results

Table 4 shows higher mortality (33 per cent) of eggs which were given zero days initial incubation and exposed to 0 C than of those which were kept at 5 C (10 per cent). Mortality was similar for the rest of the eggs. Eggs incubated initially for zero to three days did not take up water during their exposure to low temperatures, but did so eventually during final incubation at 29 C within seven days. Only some eggs which were given an initial incubation of four to six days completed water uptake before being exposed to low temperatures. After a seven day initial incubation period all eggs were fully swollen with water. Hatching of eggs without initial incubation and for both low temperature treatments started on the 13th day and continued up to the 18th day of incubation. Maximum hatching occurred, however, on the 14th day of incubation. All the hatching of eggs which were incubated initially for 2 to 11 days, occurred

within a range of two to three days; for the longer initial incubation, however, this range was extended to a period of six to seven days. The period to maximum hatching decreased from 14 to 10 days as the initial incubation increased from zero to seven days. It seems from this difference of four days in these incubation periods that eggs without low temperature treatment developed at 22 C in seven days to the stage they had reached at 29 C in four days. Thus the total incubation period at 29 C for all the post-diapausing eggs is almost always 13 to 15 days. After more than seven days initial incubation maximum hatching took place on the 10th day (sometimes on the 9th day) of final incubation. Eggs which were incubated initially for 16 and 21 days showed a somewhat lower total percentage hatch than those given 1 to 11 days initial incubation. They also showed a wide range of hatching (six to seven days) and hence a higher mean incubation period. This suggests that eggs which were kept at 22 C for longer periods before chilling might have undergone diapause more strongly than eggs which were exposed to low temperature earlier. They, therefore, might require longer low temperature treatment to break diapause. Analysis of variance showed that the variation in per cent hatch among treatments is significant at the one per cent probability level due to the interaction of low temperatures and initial incubation periods. Variation in per cent hatch among treatments of initial incubation periods, however, is significant only at the five per cent level.

#### Effects of Low Temperature on Diapausing Eggs

*N. fasciatus* is found in places where winter temperatures differ widely, it follows that this cricket possesses some mechanism by which diapause is terminated by locally available low temperatures. Lees (1955) found that diapause termination in insects from warmer localities does not require such low temperatures as in insects inhabiting colder environments. Danilyevsky (1965) and Masaki (1961) consider that genetical differentiation for terminating diapause by local low temperatures is often involved in insects with a seasonal rhythm of development adapted to different climatic areas. Bigelow (1960, 1962) reported variations in adaptability to local low temperatures in local populations of field crickets.

I was interested, therefore, in determining the range and periods of low temperatures effective in breaking diapause.

#### Methods and materials

Eight hundred eggs, which had been laid over a 24 hour period during September 1964 were divided into 16 groups of 50. All groups were incubated initially at 22 C for 10 days to allow them to reach the diapause stage, so that mortality would be lessened. Four groups each were then kept at the following temperatures: 0 C, 5 C, 10 C, and 15 C. After 15, 30, 45 and 60 days one group of eggs from each room was incubated at 29 C. The same procedure was repeated in 1965 under similar conditions.

#### Results

The percentage hatching in 11 days (the normal incubation period for post-diapause eggs incubated previously for 10 days) is plotted against

time of exposure to low temperatures in fig. 5. All four temperatures broke diapause, but forty-five day exposures were needed before this approached completeness. Frequency distributions of the percentage hatching of eggs kept for 15, 30, 45, and 60 days at 5 C (fig. 6) show that the range of hatching period decreases with increase in time of low temperature treatment. It was found by analysis of variance that the highly significant variation in hatching resulted from different durations of low temperature treatment, whereas, variations due to different low temperatures and due to the interaction of low temperatures with durations are not significant.

TABLE 4. The effect of exposure to low temperature on mortality and termination of diapause in eggs of *N. fasciatus* kept at 22 C for different periods after being deposited. Incubation temperature was 29 C.

Days of initial incuba- tion at 22 C	0 C			5 C		
	Mean in- cubation period in days at 29 C	Per cent hatch without diapause	Per cent mortality	Mean in- cubation period in days at 29 C	Per cent hatch without diapause	Per cent mortality
0	14.0	52 <sup>1</sup>	33	13.5	89	10
1	13.5	91	7	12.9	89	5
2	12.6	95	3	12.4	90	3
3	12.0	98	2	11.8	95	3
4	11.3	97	2	11.4	94	1
5	10.8	94	5	11.1	95	4
6	10.6	93	5	11.3	93	4
7	10.0	87	11	10.0	94	0
8	9.9	96	3	10.0	97	0
9	9.4	96	2	9.9	94	1
10	9.3	96	4	9.3	98	2
11	9.8	92	3	9.4	86	5
16	10.7	74	4	10.1	86	3
21	11.5	62	8	10.6	76	6

<sup>1</sup> Some eggs hatched after very long periods.

'F' value for different initial incubation periods = 3.13\*  
 5% probability level = 2.60  
 1% probability level = 3.96

'F' value for interaction of low temperatures and  
 initial incubation periods = 4.49\*\*  
 5% probability level = 2.13  
 1% probability level = 2.93

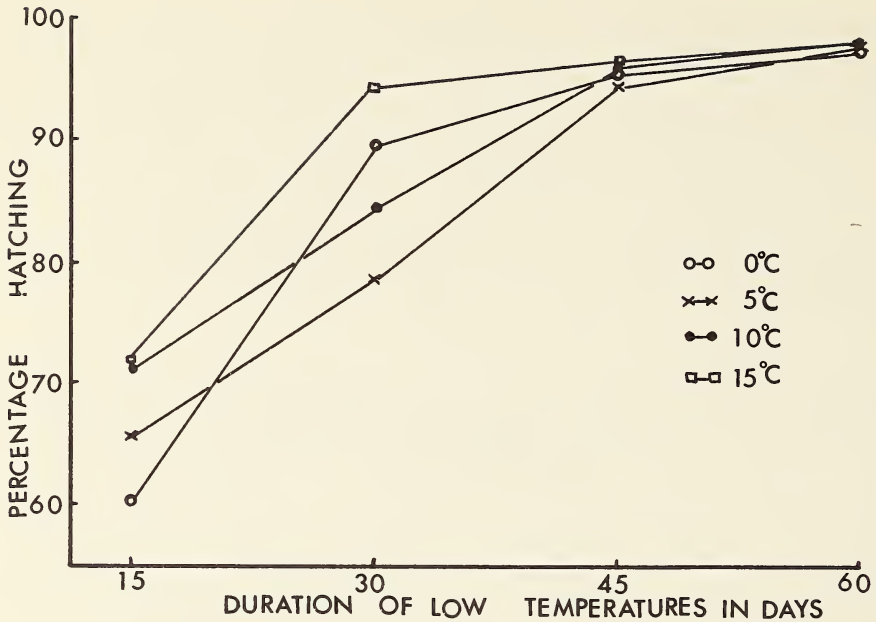


Fig. 5. The percentage hatch of diapause eggs of *N. fasciatus* after exposure to different low temperatures for different periods, prior to incubation at 29 C.

#### Effects of Low Temperature on Post-diapause Eggs

Post-diapause eggs under natural conditions sometimes experience low temperatures during development in early summer. Rakshpal (1962b) has shown that exposure of post-diapause eggs of *Gryllus pennsylvanicus* to low temperature causes some mortality. The following experiment was conducted primarily to determine whether low temperature causes any mortality in post-diapause eggs, and secondly to determine how long it takes for eggs which recover from this chilling to develop at incubation temperature.

#### Methods and materials

Six hundred eggs were counted from eggs which had been deposited within 24 hours. Two days later another similar sample of eggs was obtained for replication. Each of these samples was divided into 12 groups of 50. All the eggs were left at 22 C for 10 days and then transferred to 5 C for 30 days to terminate diapause, then brought to 29 C. Three groups (from each replicate) were then incubated continuously at 29 C to serve as controls, whereas, the other nine were moved to 5 C (second chilling) three at a time, after 4, 6, and 8 days of incubation (referred to as first post-diapause incubation). From each of these three groups one was brought to 29 C for final incubation after 5, 10, and 15 days of second chilling.



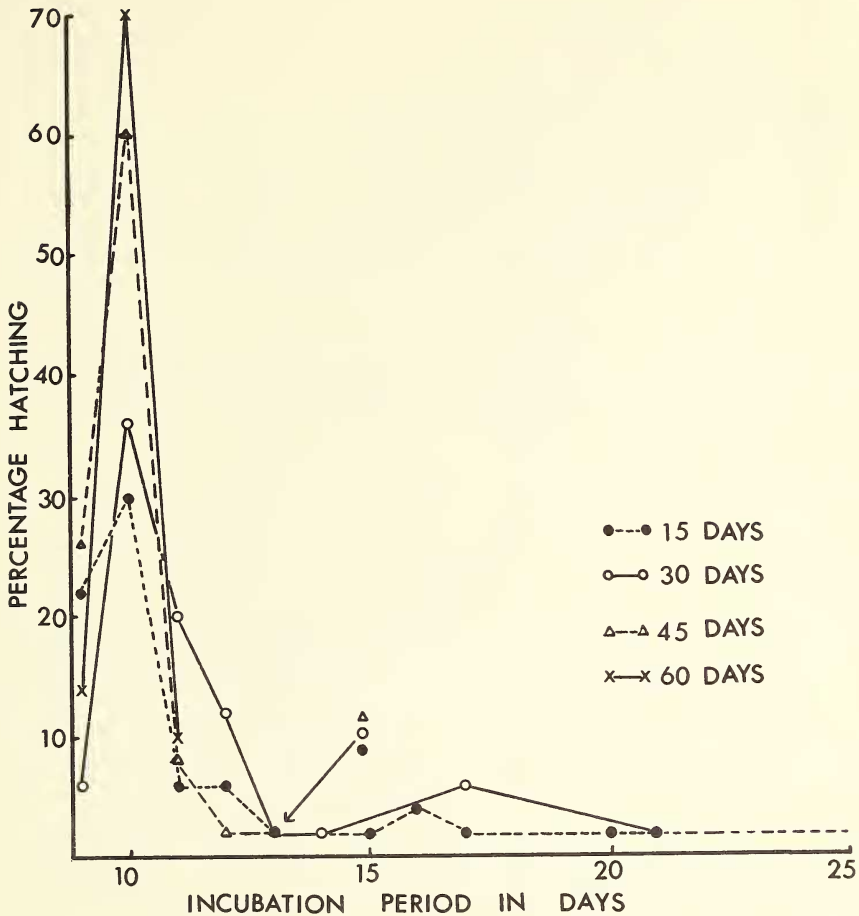


Fig. 6. Frequency distribution of hatching of eggs of *N. fasciatus* exposed to 5 C for 15, 30, 45, and 60 days and then incubated at 29 C.

#### Results

The percentage hatch at 29 C in all the treatments varied from 80 - 96. Hatch was greater in eggs which were given six days first post-diapause incubation and five days second exposure to low temperature. The control eggs started hatching on the ninth day of incubation and this was largely complete by the 11th day (normal for eggs exposed to low temperature after 10 days of pre-diapause incubation at 22 C), with maximum hatching on the 10th day. Eggs which were incubated at 29 C for first post-diapause incubation for 4, 6, and 8 days (regardless of duration of second chilling) started hatching in their final incubation after 5, 3, and 1 days with maximum hatch on the 6th, 4th, and 2nd day, respectively.

It is clear that all the groups of eggs have the same number of days of total incubation period after the termination of diapause regardless both of the time at which they were exposed to low temperature for the second time and of the duration of that exposure. Thus, once diapause is terminated development follows a simple time-temperature pattern. Exposure to low temperatures during development decreases the rate of development only. It was also observed that after the second chilling eggs recovered and resumed their normal development within 24 hours at 29 C. Statistically there is no significant variation in percentage hatch among all the treatments (including the control).

#### Effects of Alternating High and Low Temperatures

The objectives of this experiment were to determine whether the diapause-inducing influence of widely fluctuating daily temperature experienced by the females as shown in the field, can be attributed to the direct effect of these temperature fluctuations on the eggs.

#### Methods and materials

The high and low temperatures used in the experiment were 29 C and 5 C, respectively. Five hundred eggs deposited on September 13 and 500 laid on September 16 were used. Each sample was divided into five groups of 100 eggs. Four such groups from each replicate were exposed to 2, 4, 8, and 12 day cycles of high and low temperatures over a total period of 48 days. Each cycle was formed of two equal durations, one at a low and the other at a high temperature. The two day cycle of alternating high and low temperature treatment would mean one day at high and one day at low temperature alternately for 48 days. The remaining fifth group of eggs was exposed continuously to low temperature for 24 days to serve as a control. Twenty-four days was the time period chosen because, for all the treatments the total number of days spent by the eggs at low temperature was 24. After treatments eggs were incubated continuously at 29 C.

#### Results

Table 5 shows that the per cent hatch at high temperature during the treatments increased from 0 to 70 with the increase of 2 to 12 days in the duration of cycle. Some hatching occurred during the treatments because eggs remained at high temperature for a total of more than 15 days, the normal period for incubation. Furthermore, eggs which were subjected to two or four day cycles of high and low temperatures showed a very low percentage hatch when incubated continuously at the completion of treatments, that is, they were mostly in diapause. Cycles longer than four days are not as effective in inducing diapause. An analysis of variance revealed that variation in percentage hatch among different treatments was highly significant.

#### Discussion

Diapause in *N. fasciatus* is broken by low temperature even in freshly laid eggs. Lees (1955) mentioned that in many insects, particularly those of Orthoptera, exposure to low temperature should synchronize

with some definite stage in the morphological or physiological development of the embryo to break diapause. He called this stage the period of sensitivity. In *N. fasciatus* this period extends from zero to seven days at 22 C. The stages of embryogenesis in this period are all the stages up to the end of anatrepsis, which were observed by studying 40 eggs after every 24 hours from a sample of 1,000 kept at 22 C. It was found from these observations that eggs developed readily without exposure to low temperature up to the completion of anatrepsis (which comes mostly after seven days of incubation at 22 C), after which development was blocked in most of the eggs. Rakshpal (1962b) observed a similar period of sensitivity in *Gryllus pennsylvanicus* Burm.

TABLE 5. The mean percentage hatching of eggs of *N. fasciatus* during and at the end of alternating 29 C and 5 C temperature treatments for 48 days.

Duration (days) in each cycle	Per cent hatch during treatment	Per cent hatch after treatment	Total per cent hatch
2	0.0	5.0	5.0
4	4.5	16.5	21.0
8	46.5	8.5	55.0
12	70.0	12.0	82.0
48 (control)	82.0	0.0	82.0

'F' value for treatments	=	62.96**
5% probability level	=	9.28
1% probability level	=	29.46

It has been noted by Parker (1930) in *Melanoplus mexicanus* (Sauss.), Church and Salt (1952) in *Melanoplus bivittatus* (Say), and Browning (1952a, b) in *Acheta commodus* Walk., that eggs which have not reached the diapause stage but which were exposed to an adequate low temperature for an adequate period and then incubated, hatched without diapause. Browning (1952b) found that *Acheta commodus* is unusual in this respect, because in most species undergoing diapause low temperature is most effective in terminating diapause after the insects have entered diapause.

Hogan (1960a) criticized the observations of Browning from his own experiments on *Acheta commodus* but confirmed them later (1960b) by stating that exposure of pre-diapause eggs to a temperature of about 13 C so weakens the tendency of eggs to enter diapause that it is readily averted when they are transferred to a suitable incubation temperature (p. 528). In fact, in his experiments from which he criticized Browning a suitable incubation temperature was not provided for the eggs after low temperature treatment.

Only a small proportion of the pre-diapause eggs of *Gryllus pennsylvanicus* Burm. were rendered free from diapause by low temperature (5 - 7 C) and there was such a high mortality that the eggs which were incubated for one day only at 22 - 23 C before exposure to low temperature were

completely killed by the latter (Rakshpal 1962b).

The observation that eggs of *N. fasciatus* have presumably entered diapause more strongly when kept for longer periods at high temperature before low temperature treatment agrees to some extent with that of Browning (1952a) in *Acheta commodus* Walk. He has shown that a preliminary high temperature treatment for more than two days caused the eggs of *Acheta commodus* Walk. to enter diapause more firmly than if kept for a little or no time at high temperature before low temperature treatment. He believed that diapause in eggs given a lengthy initial incubation at high temperatures was more intense and so required a longer period for its breaking. Moroga (1951, cited in Lees 1955) has also observed in *Bombyx mori* L. that if eggs were given an initial incubation period ranging from 3 to 80 days, before 40 days of chilling at 5 C, the percentage of non-diapausing eggs dropped from 84 to 8.

Masaki (1962) exposed eggs of the Emma field cricket, *Gryllulus mitratus* (Burm.) to a high temperature (30 C) for different periods and then kept them at a low temperature (20 C) to determine the effect of duration of high temperature on diapause intensity. He found that eggs were most responsive to the diapause-intensifying action by 9 or 14 day high temperature treatment when they were in the late pre-diapause and early diapause stages. His findings, however, were based on the mean incubation period at 20 C after high temperature treatment. These mean incubation periods ranged from 108.5 to 132.4 days, which shows that these eggs were still diapausing.

Alternating low and high temperatures apparently cause eggs of *N. fasciatus* to undergo diapause and this effect increases as the duration of the cycle length decreases to 48 hours. This characteristic could be of high survival value by enabling the eggs laid in the late summer to undergo diapause because of low night temperatures and high day temperatures. This diapause is eventually broken by the continuous cold of winter. Rakshpal (1962a) has shown that changing high and low temperatures for 56 days, in eggs of *Gryllus pennsylvanicus* Burm. has the same effect on breaking diapause as 60 days continuous 6 to 7 C exposure. In other words, 52 days low temperature treatment (the total number of days for which eggs remained at low temperature during the treatment) in changing high and low temperatures have the same effect as 60 days continuous chilling.

In *N. fasciatus* the total number of incubation days at high temperature required in post-diapause eggs remains the same regardless of the time when these eggs were subjected to low temperature again during their incubation and the duration of that period of chilling. Thus post-diapause eggs in early summer continue to develop whenever temperatures are favourable. Low temperatures retard or prevent development and eggs resume their normal development shortly after the termination of low temperature periods. Rakshpal (1962b) has shown somewhat similar results in *Gryllus pennsylvanicus* Burm. but post-diapause eggs of this species took three days to recover from the second chilling.



## COMBINED EFFECTS OF TEMPERATURE AND PHOTOPERIOD ON DIAPAUSE

There are few papers on the effects of photoperiod on diapause in the Orthoptera. Masaki (1963) studied the effect of photoperiod on the development of *Nemobius yezoensis* Shiraki, a univoltine Japanese cricket overwintering as late instar nymphs. He found that nymphal diapause is terminated by long photoperiod (16 hours per day), but is maintained for a long time by a short one (12 hours). Other important work is that of Norris (1959, 1965) on the red locust, *Nomadacris septemfasciata* (Serv.), Woodrow (1964) on *Melanoplus devastator* Scudder, and Halliburton and Alexander (1964) on *Chortophaga viridifasciata* (DeGeer).

This study was undertaken with the hope of determining whether the univoltine life history of *N. fasciatus* at Edmonton is due to a short summer or is regulated by photoperiod causing the production of diapausing eggs. Experiments were conducted to study the effect of photoperiod on the breaking of diapause in eggs during low temperature treatment, and on the various post-embryonic stages by recording the incidence of diapause in the eggs laid by them. The criteria for diapausing and non-diapausing eggs are the same as in the previous sections.

### Influence of Photoperiod on Diapause during Chilling

Gayspitz (1953, cited in Danilyevsky 1965) found that diapause in the larvae of the pine moth, *Dendrolimus pini* L. (Lepidoptera: Lasiocampidae) under conditions of 12 hours daily light and a temperature of 20 C lasts about a month, whereas at the same temperature with continuous illumination activity begins after 17 days. Danilyevsky (1965) also mentioned a similar but stronger effect of photoperiod on the diapausing larvae of *Arctia caia* L. and *Parasemia plantaginis* L. (Lepidoptera: Arctiidae).

*N. fasciatus* deposits eggs in the soil at a depth ranging from three to seven millimeters which could be an adaptation counteracting a diapause inhibiting effect of light.

### Preliminary experiment

Five hundred eggs laid within 24 hours in September 1964 were divided into five groups of 100. Another sample of 500 eggs was used in 1965 for replication. All these eggs were left at 22 C in the dark for 10 days to allow them to reach the diapause stage. Thereafter one group from each sample was kept under 8, 12, 16, and 24 hours daily light at 5 C in light-proof compartments for 30 days. The fifth group was kept in total darkness to serve as a control. The light was provided by a 16 inch 15 w fluorescent "cool white" lamp operated by a time switch. The light intensity was 85 - 90 foot-candles on the shelf holding the petri dishes containing the eggs. All the eggs were incubated at 29 C after treatment.

**Results** - The eggs in all the treatments were virtually free from diapause. Although the difference in percentage hatch among the treatments was not significant, eight hours daily photoperiod showed slightly greater hatch (93 per cent) than the rest of the photoperiods (average

87.5 per cent). Thus it appears that low temperature alone is sufficient to break diapause in this species.

#### *The Effect of Incubation Temperatures*

It was mentioned previously that the percentage hatch of eggs of *N. fasciatus* is greater at higher incubation temperatures than at lower ones, when they are incubated without any low temperature treatment to break diapause. Moreover, eggs remain at low temperatures for six months at Edmonton. The effect of very long exposure of these eggs to low temperatures and their incubation at various temperatures to determine the degree to which diapause is terminated was therefore studied. Secondly, as pointed out in the results of the previous experiment, the effect of eight hours daily photoperiod during chilling needed further clarification. This experiment was designed to determine the effects of the above mentioned factors and their interactions.

*Methods and materials* - In this experiment two replicates each of 2,475 eggs were used. For this purpose, 300 crickets were collected on September 10, 1964 and kept in 10 battery jars in the laboratory. Eggs for one replicate were collected from those deposited on September 11. These eggs were divided into 75 groups of 33. They were kept in the dark at 22 C for 10 days and thereafter moved to 5 C. At this temperature 15 such groups were subjected to each of 0, 8, 12, 16, and 24 hours daily light. Three groups were removed each time from every sample of 15 groups after 6, 12, 24, 48, and 96 days. One group from each of these three was incubated at 29 C, 24 C, and 20 C. This experiment was repeated on the same dates in 1965 and under the same conditions to replicate for analysis of variance. Unfortunately the temperature in the cabinet set for 20 C started fluctuating so widely when the experiment was already in progress during both years that the data for 20 C had to be excluded from the statistical analysis. An analysis of variance for a split plot design was used because eggs were subjected to different incubation temperatures in different cabinets.

Those eggs which hatched within 16 days of incubation at 24 C were taken as non-diapausing since 14 to 16 days is the average incubation period at 24 C for post-diapause eggs which have been subjected to low temperature after 10 days of an initial incubation at 22 C temperature.

*Results* - Table 6 shows that the per cent hatch for different photoperiods at each of the two incubation temperatures for the same durations of chilling are not significantly different. Secondly, hatch increases with the increase in duration of low temperature treatment at both the incubation temperatures. At 29 C however, there is rapid increase in percentage hatch up to 24 days of chilling when eggs are virtually free of diapause. For chilling durations longer than 24 days the percentage hatch at this temperature does not vary much. On the other hand incubation at 24 C shows very little breaking of diapause by treatments up to 24 days at low temperature. Diapause is terminated at an incubation temperature of 24 C to a great extent when eggs had been chilled for 48 days. Ninety-six days chilling enabled almost all the eggs to develop readily at this

temperature. This means that the lower the incubation temperature the longer the low temperature treatment required to allow the eggs to develop readily.

TABLE 6. The mean percentage hatch of eggs after exposure to different photoperiods at 5 C for different periods and then incubated at 24 or 29 C.

Exposure to 5 C in days	Hours light per day at 5 C									
	0		8		12		16		24	
	24 C	29 C	24 C	29 C	24 C	29 C	24 C	29 C	24 C	29 C
6	3.0	60.0	0.0	57.0	4.0	64.0	3.2	79.5	3.2	74.2
12	3.0	82.0	22.2	80.2	18.0	95.0	30.0	82.0	36.5	95.0
24	26.2	93.0	31.0	95.0	28.0	97.0	43.0	85.5	34.5	87.0
48	83.0	93.0	72.0	83.0	75.0	96.0	93.5	98.0	84.0	96.0
96	92.0	97.0	100.0	100.0	99.0	97.0	93.2	94.0	90.0	94.2

'F' value for different low temperature durations = 114.47\*\*

5% probability level = 2.87

1% probability level = 4.43

'F' value for different incubation temperatures = 23.41\*\*

5% probability level = 4.23

1% probability level = 7.72

The observations from the previous experiment that photoperiod during chilling does not affect termination of diapause are confirmed from this experiment, because statistically there is no significant difference among hatching of eggs given different photoperiods. The only significant variations in hatching among the different treatments are those which are due to different durations at low temperature, and due to different incubation temperatures. Both these variations are highly significant. It was found that development in eggs proceeded normally at  $20 \pm 3$  C when chilled for 96 days. It is possible, therefore, that eggs after such a long chilling are capable of developing promptly at lower incubation temperatures. This confirms that no influence of photoperiod on diapause or development can be shown and that eggs are rendered free from diapause by low temperature alone. Secondly, eggs need longer exposure to low temperature for termination of diapause if they are to develop subsequently at lower incubation temperatures.

#### The Influence of Light on Nymphs

There is much evidence showing the influence of photoperiod on certain stages in the life history of different insects affecting diapause either in the succeeding stages or in the next generation. Dickson (1949) observed in *Grapholita molesta* (Busck.) in southern California that a substantial proportion of the larvae entered diapause in the early autumn when temperatures were still favourable for development. He found that dia-

pause in these larvae had already been determined by the photoperiod experienced during the early larval instars.

Danilyevsky (1948, cited in Danilyevsky 1965) found that if *Acronycta rumicis* L. (Lepidoptera: Noctuidae) is exposed during the larval feeding period to a short day-length (6 - 15 hours) virtually every individual enters diapause in the pupal stage, whereas the incidence of diapause fell to zero when the larvae experienced 17 hours daily photoperiod.

Kogure (1933) showed in *Bombyx mori* L. that light and temperature acting on eggs and early larval instars induced a particular pattern of diapause which appeared in the eggs laid by the adults arising from these stages.

In all of the previous experiments on the influence of photoperiod on diapause in *N. fasciatus* only the egg and adult stages were considered. An experiment was planned to determine whether photoperiod acting on the nymphs has any effect on the proportion of diapausing eggs deposited by the females arising from these nymphs.

#### *Methods and materials*

Newly hatched nymphs for this experiment came from eggs which were deposited in September 1965 held after diapause at 24 C. They were divided into 85 groups of ten, within 24 hours after hatching. Each group was raised to maturity on rabbit pellets in a rearing jar. Five such jars were exposed to 12 hours or 16 hours daily light in two growth chambers for each of the nymphal instars and the adult stage. The light intensity on the surface holding the jars was approximately 500 foot-candles and the temperature was kept at 24 C. Since the mean durations of the six instars at 24 C are 5.8, 5.6, 4.7, 6.3, 8.1 and 9.8 days, respectively, the first instar was exposed for six days only, the second for six days only and so on to either 12 or 16 hours daily light. Five jars were subjected to each of 0, 12, and 16 hours daily light for all the post embryonic stages, to serve as controls. All the jars were observed once a week to change the water vial, jars on a dark regime under a 40 watt red light. High mortality during handling resulted from this technique. After reaching the adult stage all the five samples of each treatment were transferred to a battery jar for convenience in getting eggs. For oviposition wet soil was provided in petri dishes in the usual manner on every alternate day and sometimes after every fourth day to minimize the exposure of adults to red light. Eggs thus obtained were sieved in water and incubated directly at 29 C.

#### *Results*

Table 7 shows the sex ratio in each treatment and the percentage hatch of the eggs at 29 C laid by the adults resulting from each treatment. It is apparent that neither of the photoperiods influences the laying of diapausing eggs since the percentage hatch of the eggs laid by the control crickets reared in darkness throughout the post-embryonic part of the life history is not much different from the other treatments. Only a small proportion of the 50 nymphs in each treatment reached maturity, which decreased the precision of the results.



TABLE 7. The influence of photoperiod on different post-embryonic stages expressed as the percentage hatching of eggs laid by the resulting females. Incubation temperature was 29 C.

12 hours daily photoperiod			16 hours daily photoperiod		
Stage illuminated	Sex		Stage illuminated	Sex	
	♂	♀		♂	♀
Per cent hatching at 29 ± 1 C of their eggs			Per cent hatching at 29 ± 1 C of their eggs		
instar 1	6	5	21.7	instar 1	8 7 51.3
instar 2	5	7	30.0	instar 2	13 16 48.0
instar 3	11	8	43.0	instar 3	9 6 44.2
instar 4	10	8	48.5	instar 4	7 15 43.3
instar 5	9	8	46.0	instar 5	12 5 28.3
instar 6	8	9	45.2	instar 6	8 13 27.7
adult	5	15	41.7	adult	5 16 23.5
all post-embryonic stages	2	5	37.0	all post-embryonic stages	6 8 21.0

Control crickets reared in total darkness yielded 7 males and 5 females and 25% of their eggs hatched.

## Discussion

Marcovitch (1923, cited in Danilyevsky 1965) working with aphids was probably the first to discover the effect of photoperiod on the life cycle of an insect. It is only during the last few years that the subject of photoperiodism has attracted close attention. In most of the insects worked on so far, diapause has been found to be influenced by photoperiod. But Danilyevsky and Gayspitz (1948, cited in Lees 1955) found that diapause in *Phalera bucephala* (L.) and *Spilosoma menthastris* Esp. (Lepidoptera) was unaffected by photoperiod. Dickson (1949) also observed no effect of photoperiod on adult diapause in *Listroderes obliquus* Klug. (Coleoptera) when larvae were grown under conditions of 9, 15, and 24 hours daily light. He also found *Lucilia sericata* Meig. (Diptera) independent of photoperiod for its diapause. Egg diapause in *N. fasciatus* also is unaffected by photoperiod in any stage.

Browning (1952b) observed that in also *Acheta commodus* Walk. that the incubation temperature influenced the termination of diapause after low temperature treatment. Exposure for 30 days to 10.3 C gave 84 per cent hatch at 29.9 C, whereas following the same low temperature treatment only 64 per cent hatched at 26.5 C and none at 20.9 C.

The observation that *N. fasciatus* is neutral to photoperiod, however, should not be taken as valid for this species in general, because only the Edmonton strain is involved. For the generalization of these observations, experiments with strains from other parts of its range will be required.



## GENERAL DISCUSSION

*N. fasciatus*, like many other Orthoptera, undergoes diapause at the close of anatrepsis. Wide variations were observed in the proportion of diapausing eggs among eggs laid during succeeding summer weeks in association with wide fluctuations in the daily range of temperature. The diapause-inducing effect of alternating high and low temperatures on the eggs in the laboratory suggests that this association is causal, and that the eggs during the pre- and post-oviposition period are affected directly by external temperatures. To resume development diapause eggs of *N. fasciatus* require to be subjected to temperatures below 10°C for periods up to 96 days followed by temperatures above 20°C. The induction of diapause by fluctuating temperatures enables most eggs to enter diapause by the end of summer. Untimely hatching of eggs in the same summer is thus greatly reduced, an adaptation of high survival value. Lees (1955, p. 29) states that as a general rule high temperatures tend to avert diapause while low temperatures favour arrest of development. This holds good in *N. fasciatus* in which a high percentage hatch would not normally occur in the latitude of Edmonton because temperatures in late summer rarely rise above 25°C. None of these processes show evidence of being affected by photoperiod or changes in photoperiod.

A second probable factor contributing to variations in the proportion of diapausing eggs, is the age of the adult female at the time of oviposition. It has been observed in *Bombyx mori* L. (Kogure 1933), *Phlebotomus papatasi* Scop. (Roubaud 1935), *Locustana pardalina* Walk. (Matthee 1954), *Gryllus pennsylvanicus* Burm. (Rakshpal 1962a) that the physiological condition of the female at the time of oviposition affects the presence or absence of diapause in the next generation. The incidence of diapause in the eggs seems to follow a normal curve of frequency distribution through adult life, but this is partially suppressed when the insects are kept in continuous darkness.

The only difference observed between diapausing and post-diapausing eggs of *N. fasciatus* was that the latter were clearer in their consistency. It is a general belief that during diapause some physiological changes occur which ultimately result in the resumption of active development. The difference in the consistency of diapausing and non-diapausing eggs in *N. fasciatus* is probably due to some such physiological changes in the egg during low temperature treatment. Zolotarev (1947 and 1950, cited in Danilyevsky 1965) calls these changes the diapause processes. Andrewartha (1952) introduced the term "diapause development" for the same thing. His term has since been used by other authors (Browning 1952a, b, Lees 1955, Masaki 1962, Beck and Alexander 1964a, b). Danilyevsky prefers the term "reactivation" for the same phenomenon, because he thinks that the term development is commonly linked with progressive growth and differentiation. Probably in these physiological changes different processes are involved in different insects (Harvey 1962).

It seems that *N. fasciatus* does not need an adaptive mechanism such as a very intense obligatory diapause or diapause decided by the photoperiod to survive in this part of the world. Diapause is facultative, be-

cause a certain percentage of the eggs always hatch providing the incubation temperature is above 26 C. Changes in temperature are the main influence determining the presence or absence of diapause in the eggs. Under natural conditions, however, univoltinism is maintained, firstly mainly by the induction of diapause through alternations of quite low temperatures at nights with fairly high temperatures during days for most of the oviposition period, and secondly by temperatures in September and October too low for the completion of development before winter. Thus even those eggs which need only a short period of low temperature for breaking diapause do not develop until June and July of the following summer when temperatures are high enough for a long enough time to complete development. By that time diapause will be broken in all the eggs. There is, therefore, almost synchronous hatching in the first half of July in nature.

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